

Supplemental Data

Mutations in *NSUN2* Cause Autosomal-

Recessive Intellectual Disability

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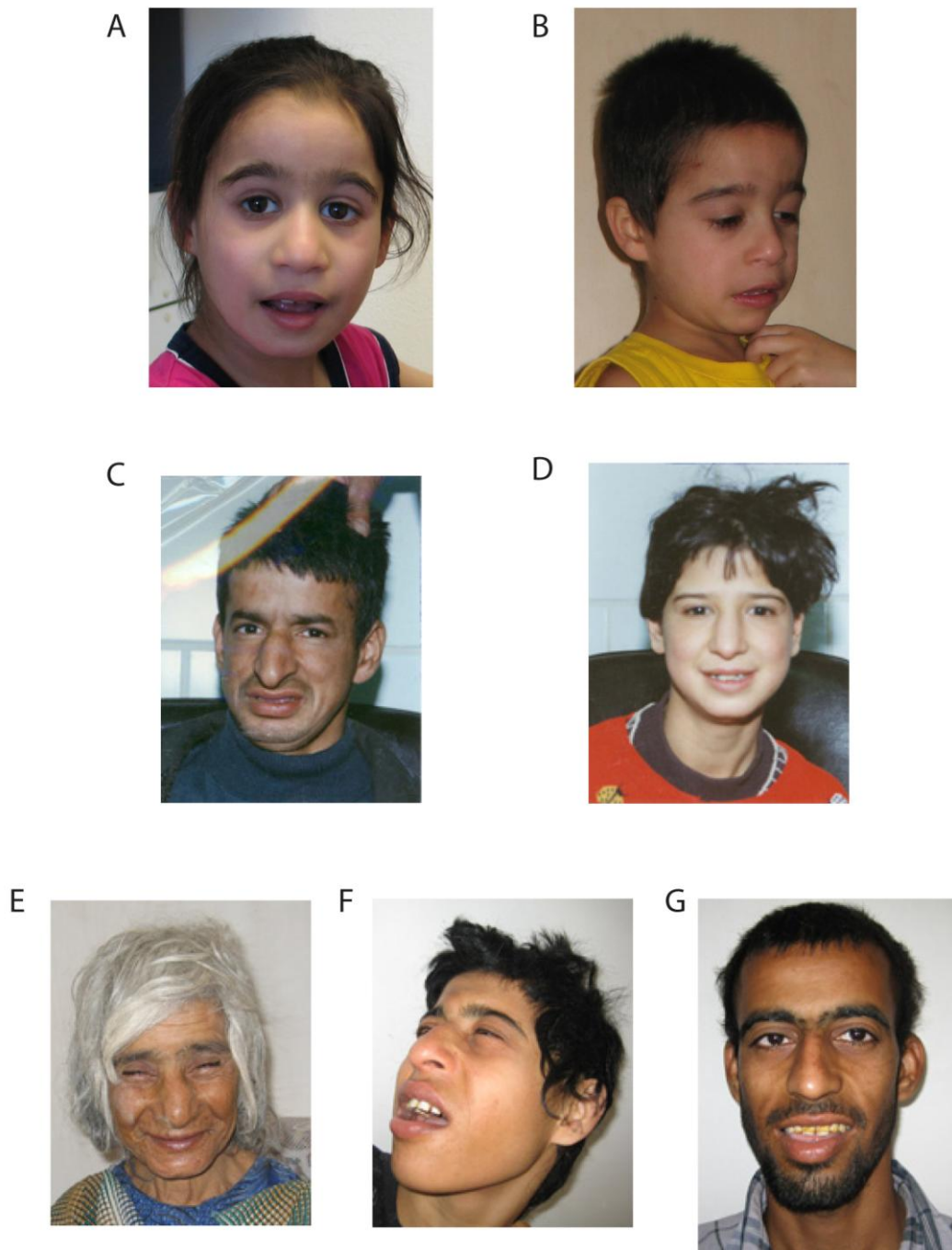


Figure S1. Facial Dysmorphism in Affected Individuals Consisting of Long and Narrow Face, Bushy Eyebrows with Synophrys, Hypotelorism, Large Nose with Long Columella and Hypoplastic Alae Nasi, Short Philtrum and Full Upper Lip

(A) Individual IV:5 of family G-013.

(B) Individual IV:6 of family G-013.

(C) Individual IV:6 of family M-192

(D) Individual IV:5 of family M-192.

(E) Individual V:1 of family M-314.

(F) Individual VI:4 of family M-314.

(G) Individual VI:7 of family M-314.

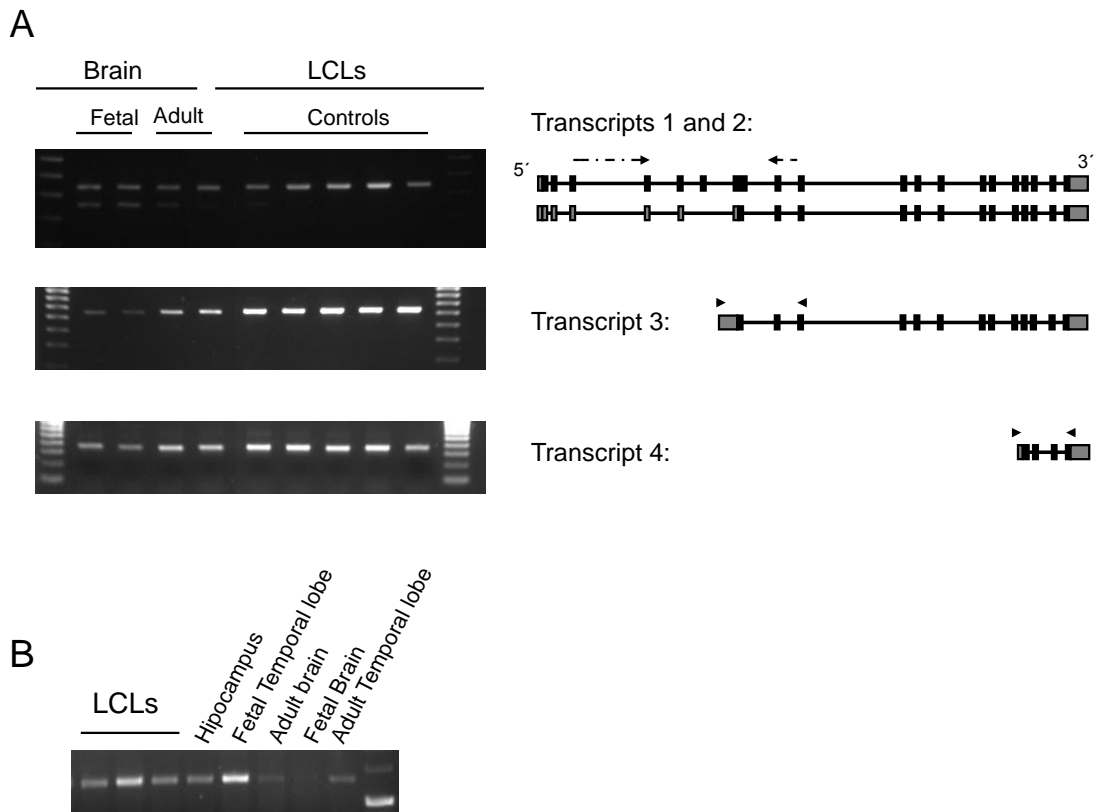


Figure S2. Expression of NSUN2 in Human Tissues

(A) RT-PCRs using transcript-specific primers (locations indicated by arrows in the schematic representations of the transcripts) show the presence of transcripts 1, 3 and 4 in RNA extracts from fetal and adult brain as well as lymphoblastoid cells (LCL). Transcript 2 could only be amplified from fetal brain RNA.

(B) RT-PCRs using primers specific for transcript 1 and 2 show the presence of transcript 1 in all tested tissues.

NSUN2 (human)	1	M	G	R	R	S	R	G	R	R	L	Q	Q	Q	Q	R	P	E	D	A	E	D	G	A	E	G	G	G	R	Q	G	E	A	G	W	E	G	G	Y	P	E	40	
NSUN2 (Drosophila)	1	M	G	R	N	Q	K	Q	N	F	F	A	A	R	K	R	-	Q	K	R	E	N	G	P	K	R	T	D	R	Q	A	Q	P	-	-	-	-	-	-	Y	E	E	34
NSUN2 (human)	41	I	V	K	E	N	K	L	F	E	H	Y	Y	Q	E	L	K	I	V	P	-	E	G	E	W	G	Q	F	M	D	A	L	R	E	P	L	P	A	T	L	R	79	
NSUN2 (Drosophila)	35	I	K	R	D	N	A	F	I	K	Y	Y	Q	L	Q	K	I	C	A	T	D	E	E	W	T	Q	F	L	A	S	I	R	D	N	L	P	T	T	F	R	74		
NSUN2 (human)	80	I	T	G	Y	K	S	H	A	K	A	I	L	H	C	L	K	N	-	-	-	K	Y	F	K	E	L	E	D	L	E	V	D	G	-	Q	K	V	E	R	114		
NSUN2 (Drosophila)	75	V	T	G	F	K	D	E	A	K	A	L	L	S	I	I	E	T	Q	L	F	T	E	Y	V	R	A	V	A	E	L	H	Q	K	A	P	E	D	V	E	R	114	
NSUN2 (human)	115	P	Q	P	L	S	W	Y	P	E	E	L	A	W	H	T	N	L	S	R	K	I	L	R	K	S	P	H	L	E	K	F	H	Q	F	L	V	S	E	T	E	154	
NSUN2 (Drosophila)	115	P	L	C	L	P	W	Y	P	N	G	L	A	Y	Q	L	H	L	T	R	K	D	I	R	R	S	E	P	L	Y	R	L	H	N	F	L	I	V	E	T	154		
NSUN2 (human)	155	S	G	N	I	S	R	Q	E	A	V	S	M	I	P	P	L	L	N	V	R	P	H	H	K	I	L	D	M	C	A	A	P	G	S	K	T	T	Q	L	194		
NSUN2 (Drosophila)	155	A	G	G	I	S	R	Q	E	A	V	S	M	I	P	P	I	V	L	D	V	R	P	T	D	K	V	L	D	M	C	A	A	P	G	S	K	T	A	Q	L	194	
NSUN2 (human)	195	I	E	M	L	H	A	D	-	M	N	V	P	F	P	E	G	F	V	I	A	N	D	V	D	N	K	R	C	Y	L	V	H	Q	A	K	R	L	S	S	233		
NSUN2 (Drosophila)	195	I	E	A	L	H	A	A	P	E	E	H	K	I	P	P	G	F	V	L	A	N	D	V	D	N	N	R	C	Y	M	L	V	H	Q	A	K	R	L	S	234		
NSUN2 (human)	234	P	C	I	M	V	V	N	H	D	A	S	S	I	P	R	L	Q	I	D	-	V	D	G	R	K	E	I	L	F	Y	D	R	I	L	C	D	V	P	C	S	272	
NSUN2 (Drosophila)	235	P	C	L	L	T	N	H	D	S	S	V	F	P	N	L	Q	I	T	T	K	P	D	G	S	K	A	I	L	K	F	D	K	I	L	C	D	V	P	C	S	274	
NSUN2 (human)	273	G	D	G	T	M	R	K	N	I	D	V	W	K	K	W	T	L	N	S	L	Q	L	H	G	L	Q	L	R	I	A	T	R	G	A	E	Q	L	A	E	312		
NSUN2 (Drosophila)	275	G	D	G	T	L	R	K	N	P	D	I	W	L	K	W	N	L	A	Q	A	Y	N	L	H	G	I	Q	Y	R	I	V	R	R	G	A	E	M	L	E	V	314	
NSUN2 (human)	313	G	G	R	M	V	Y	S	T	C	S	L	N	P	I	E	D	E	A	V	I	A	S	L	L	E	K	S	E	G	A	L	E	L	A	D	V	S	N	E	L	352	
NSUN2 (Drosophila)	315	G	G	R	L	V	Y	S	T	C	S	L	N	P	I	E	N	E	A	V	L	Q	R	I	I	K	D	A	D	G	A	L	E	L	V	D	A	G	H	L	V	354	
NSUN2 (human)	353	P	G	L	K	W	M	P	G	I	T	Q	W	K	V	M	T	K	D	-	G	Q	W	F	T	D	W	D	A	V	P	H	S	R	H	T	Q	I	R	P	T	391	
NSUN2 (Drosophila)	355	P	G	L	K	Y	K	P	G	M	T	D	W	K	L	M	T	K	E	V	D	Q	I	F	T	R	F	E	V	P	E	S	L	H	T	I	I	R	P	G	394		
NSUN2 (human)	392	M	F	P	P	K	D	P	E	K	L	Q	A	M	H	L	E	R	C	L	R	I	L	P	H	H	Q	N	T	G	G	F	F	V	A	V	L	V	K	K	S	431	
NSUN2 (Drosophila)	395	M	F	P	L	P	-	A	D	E	M	A	K	I	G	L	E	K	C	L	R	V	L	P	H	L	Q	D	S	G	G	F	F	V	A	V	L	E	K	R	R	433	
NSUN2 (human)	432	S	M	P	W	N	K	R	Q	P	K	L	Q	G	K	S	A	E	T	R	E	S	T	Q	L	S	P	A	D	L	T	E	G	K	P	T	D	P	S	K	L	471	
NSUN2 (Drosophila)	434	Q	L	S	F	E	K	N	D	V	V	E	L	V	K	L	N	E	T	A	K	Q	P	A	A	E	P	Q	V	D	A	D	G	K	P	I	E	-	-	-	469		
NSUN2 (human)	472	E	S	P	S	F	T	G	T	G	D	T	E	I	A	H	A	T	E	D	L	E	N	N	G	S	K	K	D	G	V	C	G	P	P	S	K	K	M	K	511		
NSUN2 (Drosophila)	470	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	E	K	S	V	P	W	G	P	Q	R	K	K	R	483		
NSUN2 (human)	512	L	F	G	F	K	E	D	P	F	V	F	I	P	E	D	D	P	L	F	P	P	I	E	K	F	Y	A	L	D	P	S	F	P	R	M	N	L	L	T	R	551	
NSUN2 (Drosophila)	484	L	H	G	Y	K	E	D	P	Y	V	F	F	G	E	N	D	P	D	Y	Q	A	I	K	E	F	Y	Q	L	D	E	S	L	S	Q	R	C	L	L	T	R	523	
NSUN2 (human)	552	T	T	E	G	K	K	R	Q	L	Y	M	V	S	K	E	L	R	N	V	L	L	N	N	S	E	K	M	K	V	I	N	T	G	I	K	V	W	C	R	N	591	
NSUN2 (Drosophila)	524	C	V	T	E	K	K	K	N	I	Y	C	S	E	P	I	R	D	L	V	L	N	N	E	N	N	I	K	I	N	T	G	V	K	T	E	V	R	C	563			
NSUN2 (human)	592	N	S	G	E	E	F	D	C	A	F	R	L	A	Q	E	G	I	Y	T	L	Y	P	F	I	N	-	S	R	I	I	T	V	S	M	E	D	V	K	I	L	630	
NSUN2 (Drosophila)	564	E	N	-	R	H	T	V	H	P	F	R	L	A	Q	E	G	L	Q	T	S	N	A	F	M	G	A	-	S	R	R	I	Q	V	E	R	E	D	L	V	M	M	602
NSUN2 (human)	631	L	T	Q	E	N	-	-	-	P	F	F	R	K	L	S	S	E	T	Y	S	Q	A	K	D	L	A	K	G	S	I	V	L	K	Y	E	P	D	S	A	666		
NSUN2 (Drosophila)	603	L	N	C	T	D	P	T	Q	P	P	S	T	H	E	L	K	K	E	T	Q	E	R	C	K	E	L	G	V	G	S	C	I	L	K	Y	V	D	Q	-	640		
NSUN2 (human)	667	N	P	D	A	L	Q	C	P	I	V	L	C	G	W	R	G	K	A	S	I	R	T	F	V	P	K	N	E	R	L	H	Y	L	R	L	M	M	G	-	L	704	
NSUN2 (Drosophila)	641	-	-	-	-	R	F	T	L	Y	T	V	G	W	R	G	T	S	S	L	R	A	Y	V	Q	K	N	D	E	T	I	H	I	L	R	L	L	G	A	D	L	675	
NSUN2 (human)	705	E	V	L	G	E	K	K	Y	E	G	V	I	L	T	N	E	S	A	A	A	S	T	G	Q	P	D	N	D	V	T	E	G	Q	R	A	G	E	P	N	S	P	744
NSUN2 (Drosophila)	676	S	K	F	E	T	N	K	Y	E	D	A	R	V	A	A	A	A	A	A	D	A	E	V	G	K	S	A	E	A	E	A	D	S	S	G	D	G	D	A	T	715	
NSUN2 (human)	745	D	A	E	E	A	N	S	-	-	P	D	V	T	A	G	C	D	P	A	G	V	H	P	P	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	767		
NSUN2 (Drosophila)	716	E	S	T	F	S	G	S	G	A	I	D	V	T	V	A	A	E	T	T	G	T	P	M	D	T	E	V	V	A	T	S	-	-	-	-	-	-	-	746			

Figure S3. ClustalW Analyses of Human and *Drosophila* NSUN2

ClustalW alignment of human and *Drosophila* NSUN2. Boxed are identical (dark grey) and similar (light grey) amino acids. Blue underlined is the region of about 300 amino acids in the center of the two proteins which shows 74% of similar and 59% of identical amino acids. Asterisks highlight the two nonsense mutations found in affected individuals (p.Gln227* and p.Gln372*).

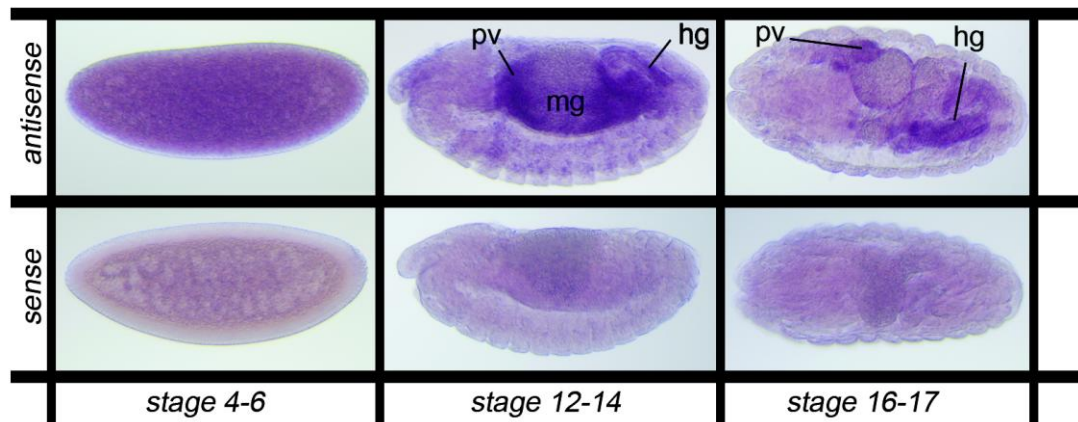


Figure S4. Expression of *Drosophila* Nsun2 mRNA with In Situ Hybridization

Dioxigenin-labeled sense and antisense probes for dNsun2 were used to visualize dNsun2 mRNA in wild-type embryos (strain w1118). dNsun2 mRNA is expressed with a maternal contribution (stage 4-6, left panel). Older embryos show a weak ubiquitous expression and distinct expression in the proventriculus area (pv) of the foregut and in the hindgut (hg) (stages 12-17). Sense controls (lower panel) stained under the same conditions as antisense (upper panel). Embryos are oriented anterior to the left. mg, midgut.

Table S1. Aversive Olfactory Avoidance and Shock Reactivity

	<u>Olfactory Avoidance</u>		<u>Shock Reactivity</u>
	OCT	MCH	
<i>Canton-S</i>	48.70 ± 2.47	51.52 ± 4.09	53.52 ± 4.32
<i>w¹¹¹⁸</i>	47.77 ± 5.66	46.03 ± 3.50	52.69 ± 5.70
<i>dNsun2^{ex1}</i>	51.61 ± 3.38	51.26 ± 5.16	54.83 ± 4.55

Male flies were tested for their innate odor and shock avoidance, which also involves elementary locomotion. Denoted are the means of the performance indices ± SEM, (n ≥ 8).